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Choroideremia Is Linked to the Restriction Fragment Length Polymorphism *DXYS1* at XQ13-21

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SUMMARY

Choroideremia (McK30310), an X-linked hereditary retinal dystrophy, causes night-blindness, progressive peripheral visual field loss, and, ultimately, central blindness in affected males. The location of choroideremia on the X chromosome is unknown. We have used restriction fragment length polymorphisms from the X chromosome to determine the regional localization of choroideremia by linkage analysis in families with this disease. One such polymorphic locus, *DXYS1*, located on the long arm (Xq) within bands q13-q21, shows no recombination with choroideremia at lod = 5.78. Therefore, with 90% probability, choroideremia maps within 9 centiMorgans (cM) of *DXYS1*. Another polymorphic locus, *DXS11*, located within Xq24-q26, also shows no recombination with choroideremia, although at a smaller lod score of 1.54 (90% probability limit $\theta < 30$ cM). This linkage with *DXS11*, a marker that is distal to *DXYS1*, suggests that the locus for choroideremia is also distal to *DXYS1* and lies between these two markers in the region Xq13-q24. These results provide regional mapping for the disease that may be useful for prenatal diagnosis and, perhaps ultimately, for isolating the gene locus for choroideremia.

INTRODUCTION

Choroideremia (McK30310) is one form of X-linked retinal dystrophy causing progressive visual impairment and eventual blindness in affected males during

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early adulthood [1, 2]. The disease is generally quite rare although the frequency has been reported to be as high as one in 40 in certain regions of Finland [3]. The gene or genes involved in choroideremia are known to be located on the X chromosome because the disease is inherited in an X-linked manner in families, but its exact location on the X and the nature of the gene defects responsible for the disease are unknown. Biochemical or molecular methods for prenatal diagnosis of choroideremia are unavailable. Genetic markers linked to the choroideremia locus constitute, therefore, the only currently feasible approach to prenatal diagnosis of this condition.

Previous attempts at mapping choroideremia by classical linkage analysis have unfortunately proved unsuccessful [4, 5]. However, the recent development of restriction fragment length polymorphisms (RFLPs) has greatly expanded the power of linkage analysis for gene mapping [6–10]. We have used X-linked RFLPs in family linkage studies in order to determine the regional localization of choroideremia and have mapped it provisionally within the region Xq13-q24 by linkage to two distinct RFLP loci: *DXS11* [11] and *DXYS1* [12].

MATERIALS AND METHODS

Family Studies

Three families, in which X-linked choroideremia segregated, were ascertained through the Cullen Eye Institute and by regional and national referral. Within each kindred, a detailed family history and pedigree were obtained through personal interviews with all available family members. A careful ophthalmological examination, with particular attention to binocular indirect stereo-ophthalmoscopy with scleral depression, was performed by R. A. L. on all available members of each kindred. Each subject, or the responsible adult on behalf of minors, signed a Consent for Participation approved by the Institutional Review Boards for Human Research at Baylor College of Medicine, The Methodist Hospital, and the Texas Children's Hospital, Houston, Texas.

The clinical criteria for establishing the diagnosis of choroideremia in an affected male included: (1) a positive family history of choroideremia compatible with X-linked transmission through three generations; (2) personal history of nyctalopia and visual field constriction present in the first two decades of life; and (3) characteristic ophthalmoscopic features of peripheral pigmentary retinal dystrophy with large, often confluent, oval or round zones of atrophy of the retinal pigment epithelium and choriocapillaris, with relative preservation of the central macular area and sparing of the inner retina, retinal vessels, and optic disc until later life.

The criteria for assigning the carrier state to a female at risk in each kindred included: (1) clear-cut genetic evidence for the carrier state, either as the daughter of a male documented to have the disease or as the mother of (at least) one affected male; and /or (2) ophthalmoscopic evidence of any of the changes previously described in carrier females involving the retinal pigment epithelium, ranging from punctate and peppery to coarse and reticular, and characteristically becoming more extensive toward the retinal periphery [1, 2, 13].

Heparinized venous blood from all available family members was used both as a source of DNA [11] and to establish permanent lymphoblastoid lines [14] in culture.

Restriction Fragment Length Polymorphisms

DNA probes pHPT30, 22-33, and pDP34 were used to detect RFLPs in restriction enzyme-digested genomic DNA from family members. Probe pHPT30 is a complemen-

tary DNA for human hypoxanthine phosphoribosyltransferase (*HPRT*) that detects a three-allele *Bam*HI RFLP at the human HPRT locus [15]. *DXS11* is a two-allele RFLP detected by probe 22-33, an anonymous 1.8-kilobase (kb) *Hin*dIII fragment reported to map within Xq24→qter. *DXS11* is, however, absent from a mouse-human hybrid containing a single human translocation chromosome t(X,11)(q25-26; q23) [16] and is therefore excluded from Xq26→qter and more narrowly localized to Xq24-q26 (our unpublished data, 1984). *DXS11* consists of two alleles, which are *Taq*I fragments of 11 kb (allele frequency = .83) and 17 kb (allele frequency = .17) [11]. *DXYS1*, mapped to Xq13-21, is a *Taq*I polymorphic locus with X-specific alleles of 12 kb (allele frequency = .4) and 11 kb (allele frequency = .6) [10] detected with a cloned 2.2-kb *Eco*RI fragment: pDP34 [12].

Southern Blot Analysis

DNA from individuals and hybrid lines was prepared by published procedures [11]. For RFLP analysis, 10 μg of DNA were digested with the restriction enzymes *Taq*I or *Bam*HI (4–8 U/μg DNA) under appropriate buffer and temperature conditions for 4 hrs to overnight, electrophoresed for 16–40 hrs at constant voltage 25–40 V through 0.8% agarose, and then transferred to nitrocellulose [17]. Radioactive probes were prepared by *E. coli* polymerase I nick-translation using [^{32}P]dCTP to $> 10^8$ cpm/μg [18]. Hybridizations were carried out for 18 hrs in 50% formamide, 5× SSPE, 1× Denhart, and 250 μg/ml denatured herring sperm DNA [19].

Linkage Analysis

Linkage analysis was conducted with the computer program LIPED [20]. To provide parameters for the LIPED program, the disease frequency was estimated to be 10^{-4} and the RFLP allele frequencies used were the previously published values [10, 11, 15]. Varying the disease frequency 10-fold in either direction had no effect on the results of the linkage analysis. The maximum likelihood recombination distance ($\hat{\theta}$) between loci was determined for each family as that value of the recombination distance (θ) that maximizes the odds ratio. Confidence limits for $\hat{\theta}$ were calculated as described by Emery [21].

RESULTS

Family XL-14 is a kindred in which choroideremia and the RFLPs for *HPRT*, *DXS11*, and *DXYS1* were all segregating (fig. 1). Meioses in individuals informative for choroideremia and *DXYS1* revealed no recombinational events. The *DXYS1* alleles seen on Southern blot are shown in figure 2 for a part of family XL-14. The X-linked inheritance of the 11-kb and 12-kb alleles from individual III-6 and the 12-kb allele from individual III-7 can be followed in their offspring IV-4–IV-8. The 14-kb *Taq*I fragment arises from the human Y chromosome, present only in males, and is unrelated to the RFLP on the X. In two other families, XL-33 and XL-34, more meioses informative for choroideremia and *DXYS1* were available. No obligatory recombinational events were seen in these families. With these data, lod scores at varying recombination distances (θ) for each family and an overall lod score were calculated as shown in table 1. If the choroideremia locus is assumed to be identical in all three families, the maximum likelihood estimate of the recombination distance ($\hat{\theta}$) between *DXYS1* and choroideremia for all three families was calculated to be 0 cM at a lod score of 5.78 (90% confidence limits $0 \leq \hat{\theta} < 9$ cM). For *DXS11*, family XL-

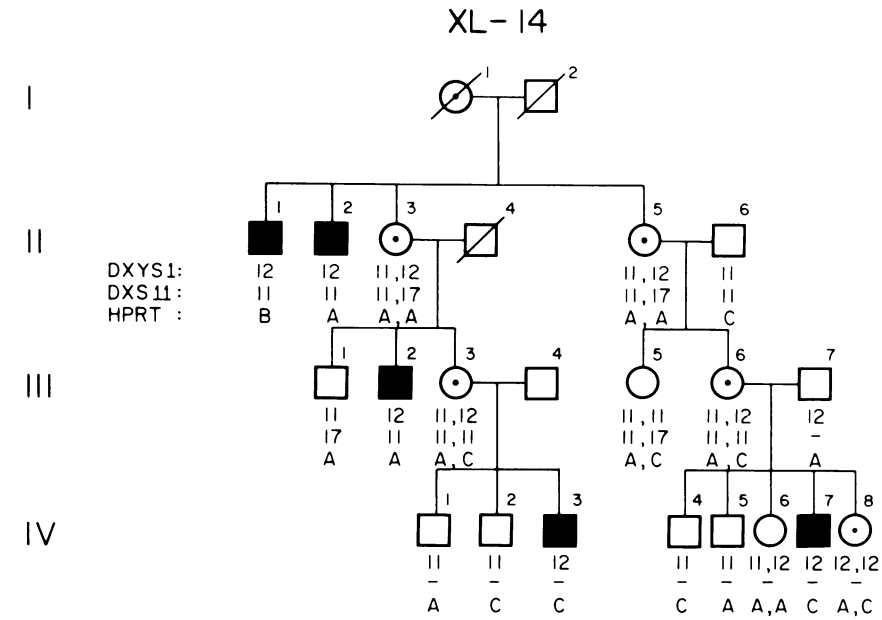

FIG. 1.—Pedigree of family XL-14 in which choroideremia and alleles for DXYS1, DXS11, and HPRT are segregating. For choroideremia, (□) is an unaffected male, (■) is an affected male,(⊙) is a carrier female, (○) is a normal female by examination. An individual's genotype, when known, at the three RFLP loci (DXYS1, DXS11, and HPRT) is given underneath the individual's pedigree symbol. For DXYS1, the alleles are 11 kb and 12 kb; for DXS11, the alleles are 11 kb and 17 kb; for HPRT, the A allele is a pair of *Bam*HI fragments of 22 kb and 25 kb, the B allele is a 12-kb/25-kb pair, and the C allele is a 22-kb/18-kb pair. Genotype unknown or not determined is represented by −.

14 alone was informative (fig. 1). In this family, θ was 0 cM between choroideremia and *DXS11* although with a substantially smaller lod score of 1.54 (90% probability limits $0 < \hat{\theta} < 30$ cM) (table 2).

In family XL-14, females heterozygous for both the *HPRT* RFLP and choroideremia were available (fig. 1). Multiple recombinational events have occurred in their offspring and, as shown in table 2, no detectable linkage between these two loci was present ($\hat{\theta} = 50$ cM, with 90% confidence limit $\hat{\theta} > 22$ cM). Thus, choroideremia showed strong linkage to *DXYS1* and measurable linkage to *DXS11* but no linkage to *HPRT* in these families.

DISCUSSION

Choroideremia (McK30310) is an infrequent, bilateral, symmetrical progressive dystrophy in males that affects the outer retina, pigment epithelium, and choroid, and is characterized by nyctalopia, visual field constriction, and eventual blindness. Although the disease was first described by Mauthner in 1872 [22], its X-linked mode of transmission and a firm description of its carrier state were not established until 1942 by Goedbloed [23] and by Waardenburg [24].

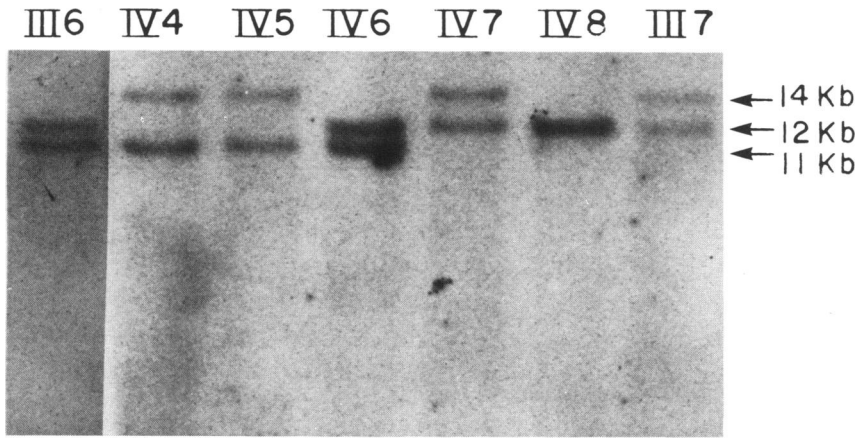

FIG. 2.—Southern blot analysis of *Taq*I-digested DNAs, probed for DXYS1, from a family within the XL-14 kindred. Each lane of DNA is labeled with the pedigree position (fig. 1) of the individual from whom the DNA was obtained. *Taq*I restriction fragments seen at DXYS1 include the X-linked RFLP alleles of 11 kb and 12 kb as well as an invariant Y-linked 14-kb fragment.

Genetic heterogeneity is considered unlikely on clinical grounds since different families with choroideremia demonstrate typical X-linked inheritance and a similar and distinctive clinical phenotype in affected males as well as a characteristic constellation of features in heterozygous females [13, 25–27]. However, in the absence of definitive molecular data, the existence of more than one choroideremia locus cannot be ruled out.

Previous attempts at mapping choroideremia with classical markers have been unsuccessful [4, 5]. With newer mapping techniques made possible by

TABLE 1

LOD SCORES AT VARYING VALUES OF RECOMBINATION DISTANCE θ BETWEEN CHOROIDEREMIA AND *DXYS1* IN THREE FAMILIES SEGREGATING FOR MARKERS AT BOTH LOCI

θ	XL-14	XL-33	XL-34	Total
0.5	0	0	0	0
0.45	.41	.013	.17	0.59
0.40	.84	.049	.33	1.22
0.35	1.28	.104	.47	1.85
0.30	1.71	.170	.60	2.47
0.25	2.12	.243	.71	3.08
0.20	2.52	.318	.82	3.66
0.15	2.91	.393	.92	4.23
0.10	3.28	.465	1.02	4.76
0.05	3.63	.535	1.12	5.28
0	3.97	.602	1.21	5.78

NOTE: Total lod scores for all three families are given in the right-hand column. $\hat{\theta}$ = 0 cM at lod = 5.78.

TABLE 2

LOD SCORES AT VARYING VALUES OF RECOMBINATION DISTANCE θ BETWEEN CHOROIDEREMIA AND *DXS11* AND BETWEEN CHOROIDEREMIA AND *HPRT* IN FAMILY XL-14

θ	XL-14 Choroideremia–*DXS11*	XL-14 Choroideremia–*HPRT*
0.5	0	0
0.45	.18	−0.15
0.4	.36	−0.33
0.35	.53	−0.55
0.3	.69	−0.71
0.25	.85	−0.83
0.2	1.00	−1.18
0.15	1.14	−2.27
0.1	1.28	−3.21
0.05	1.41	−4.90
0	1.54	∞

NOTE: $\hat{\theta}$ between choroideremia and *DXS11* is at 0 cM, with lod = 1.54. $\hat{\theta}$ between choroideremia and *HPRT* is at 50 cM.

recombinant DNA technology, we have found the locus for choroideremia to be linked to the polymorphic loci *DXYS1* (Xq13-21) and *DXS11* (Xq24-26). Since *DXYS1* and *DXS11* are cytogenetically separable on the X chromosome and since both are linked to choroideremia, one might deduce that these two RFLPs actually flank the choroideremia gene. Indeed, the strongly negative linkage results between choroideremia and *HPRT*, located at Xq26-27 [27], combined with the measurable linkage with *DXS11*, located within Xq24→26, makes the locus for choroideremia unlikely to be distal to *DXS11*. In addition, the very close linkage of choroideremia to *DXYS1*, located at Xq13-21, proximal to *DXS11*, provides more strong evidence against the locus for choroideremia being distal to the region between *DXS11* and *DXYS1*. However, although $\theta = 0$ cM between *DXS11* and choroideremia, the 90% probability limit of 30 cM is large enough to be compatible with the locus for choroideremia lying close to but proximal to *DXYS1* and not within the segment of the X chromosome between *DXS11* and *DXYS1*. Further family analysis with more RFLPs in this region should allow determination of the gene order and more accurate measurement of the recombination distances between these two RFLPs and choroideremia.

Gene mapping with RFLPs has recently been used to localize another retinal dystrophy, X-linked retinitis pigmentosa (XLRP) (McK31260), to the proximal short arm of the X chromosome (Xp11.3) by linkage to the anonymous polymorphic locus *DXS7* [8]. Although choroideremia and XLRP are both X-linked, they are distinguishable on clinical grounds both in the affected males and carrier females and therefore are considered to be different disorders. These data provide confirmatory genetic evidence that choroideremia and XLRP arise from mutations at distinct loci.

In addition to mapping the genes for human diseases, RFLPs are applied also to the study of meiotic recombination in man. Family XL-14 provides an opportunity to measure the recombination distance between two loci, *HPRT* and *DXYS1*, whose cytogenetic locations are known accurately by somatic cell hybrid and in situ hybridization techniques. In this family, the *HPRT* locus, at Xq26-27, and *DXYS1*, at Xq13-Xq21, show no measurable linkage with each other ($\theta = 50$ cM, with 90% confidence limit > 22 cM). Because the recombination length of the entire 3×10^9 base pairs (bp) of DNA in the human genome has been estimated to be 3,000 cM [9], each G-band of a trypsin G-banded human chromosome at the 450-band stage should be approximately $\cong 7$ cM in length. Since these two markers are separated by 5–7 G bands at this level of resolution, the lack of measurable linkage between *HPRT* and *DXYS1* is in accord with a recombination distance of 35–50 cM between them. Thus, even within this seemingly small region of the X, these two long arm loci are no more closely linked than if they were located on different chromosomes.

Since choroideremia is a disease of unknown etiology whose expression is limited to the eye, prenatal diagnosis has been hitherto unavailable. Linkage analysis with RFLPs constitutes, therefore, the only potential avenue for prenatal diagnosis at this time. Since the allele frequencies for *DXYS1* are 0.6 and 0.4, approximately 50% of carriers for choroideremia would be expected also to be heterozygotes at the *DXYS1* locus. The recombination distance θ between *DXYS1* and choroideremia is ≤ 9 cM with 90% probability. A female carrier for choroideremia who is also heterozygous at *DXYS1*, with the phase of the *DXYS1* alleles and the choroideremia mutation known from the pedigree, would have her risk of having an affected male modified from 50% to either $\leq$ 9% or $\geq$ 91% (using the upper 90% confidence limit for θ) depending on the genotype of the fetus at the *DXYS1* locus. This degree of uncertainty when a single linked marker is used may still be too high for clinical application. However, with two markers flanking the choroideremia locus, as *DXYS1* and *DXS11* may prove to be, double crossover events would need to occur to produce diagnostic errors. Closely linked flanking markers can play a significant role in the management of families that wish to make use of the prenatal diagnosis in pregnancies at risk for choroideremia.

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